SYNOPSIS

Ph.D in Zoology

Chitosan nanoencapsulation of garlic and turmeric essential oils for its insecticidal and antifungal activities against stored groundnut

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INTRODUCTION

Food safety and security is one of the major concerns in the present world. Preservation and safe storage of food grains are essential for their timely delivery to consumers. Groundnut (*Arachis hypogaea* L.), belonging to the family leguminaceae, is one of the dominant oilseed crops of many sub-tropical and tropical countries. In India, groundnuts are stored as both pods and kernels. Both forms are infested by groundnut bruchid, *Caryden serratus* Olivier (Bruchidae: Coleoptera), a major pest of stored groundnut causing quantitative and qualitative losses (Harish *et al*., 2014). *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), a red flour beetle, is a (2.3–3.5 mm) universally important postharvest insect pest that infected a wide range of stored grain commodities. Furthermore, *T. castaneum* secreted toxins, namely quinones, by leaving a persistent and dangerous to humans. Besides, stored grain insect pests can increase the moisture content level for postharvest grain, promoting the proliferation of microbial growth (Cui *et al*., 2021) and storage of foods, threatening the development of food industries and consumers. In the food industry, *Aspergillus flavus* and its byproducts, especially aflatoxin B1 have received considerable attention among other molds due to their potential to cause cancer, teratogenicity, and immunosuppression (Li *et al*., 2019). Consequently, the accumulative impact of fungal infection may significantly influence food products and human health.

Food industries are focusing on developing unique botanical substances-based preservatives, particularly aromatic and medicinally important plants extracted essential oil and their secondary active components. Previously, several researchers suggested that EOs have significant antibacterial, antifungal, anti-mycotoxicgenic, and antioxidants properties in various postharvest food storage systems. Despite EOs and terpene having numerous beneficial properties, their antimicrobial effects in the food system is limited because of their insolubility in water, volatility, quick degradation, and deterioration when air exposure. Nanoencapsulation of EOs and their significant constituents is a practical approach to protect them from degradation under adverse environmental conditions and increase stability.

Allium sativum (L.) (garlic) belongs to the family of Amaryllidaceae and is mainly composed of diallyl tetrasulfide, allyl methyl trisulfide, allyl (E)-1-propenyl disulfide, allyl trisulfide, and allyl disulfide. Previously, Teixeira *et al*. (2014) observed that sulfide compounds of garlic EO were binding with hydrogen sulfide of the cell and producing disulfide bonds, exhibiting antibacterial against *Brochothrix thermosphacta*, *Escherichia coli*, and *Listeria innocua*. Despite EOs and terpene having numerous beneficial properties, their

application is limited because of their insolubility in water, volatility, quick degradation, and deterioration when exposed to air. *Curcuma longa* (L.) (Turmeric) belongs to the family of Zingiberaceae and is distributed in tropical Southeast Asia, Southern India, and Indonesia. Sesquiterpenoids and monoterpenoids are the major compounds of the Curcuma species. Recently, *C. longa* rhizome oil has been reported as anti-inflammatory, antihyperlipidemic, antioxidant, antiplatelet, antiarthritic, and cardiovascular protection activity (Li *et al*., 2019).

Nanoencapsulation of EOs and their significant constituents is a practical approach to protect them from degradation under adverse environmental conditions and increase stability. Chitosan (CS), a linear copolymer made of N-acetyl-D-glucosamine and (1-4) linked D-glucosamine units, is a significant raw material in pharmacological, medical, and crop management (Mondejar-Lopez *et al*., 2022). Due to their distinctive qualities, which include abundance, biocompatibility, hydrophilicity, biodegradability, non-toxicity (GRAS status), excellent film, gel, and particle-forming properties, and exceptional antifungal activity, it has been regarded among the most capable wall materials for EO nanoencapsulation and bioactive materials.

Among the several methods proposed to prepare to encapsulate, the ionic gelation approach has received much attention among the methods used to create chitosan nanoparticles since it produces stable nanoparticles while being non-toxic, convenient, and free of organic solvents. These techniques, interaction of an anionic cross-linking agent like sodium tripolyphosphate with cationic polymers like chitosan, gelatin, and dextran (S-TPP), which is the most effectively used cross-linking agent due to its multivalent and non-toxic properties (Chaudhary *et al*., 2021). In addition, it discovered antibacterial and antifungal activities, seed coating agents, insecticidal and repellency activity, and packaging materials.

Therefore, the present study to emphasized the preparation and characterization of GEO and TEO within chitosan nanoparticles (GEO-CSNPs and TEO-CSNPs) to improve their stability and investigate their insecticidal efficacy and *In vitro* and *In situ* antifungal activity.

In view of the above, the current investigation was undertaken with the following objectives.

- To analyze the chemical composition of garlic and turmeric essential oils
- To prepare the essential oils loaded chitosan-STPP nanoparticles and to characterize them using UV- vis spectrophotometer, FT-IR, TGA, XRD, DLS, Zeta potential and TEM

- To assess the impact of GEO, GEO-CSNPs, TEO and TEO-CSNPs on the insecticidal, antifeedant and repellent activities against *C. serratus* and *T. castaneum*
- To evaluate the effect of GEO, GEO-CSNPs, TEO and TEO-CSNPs on the enzymatic antioxidants in *C. serratus* and *T. castaneum*
- To evaluate the growth regulator gene expression of GEO-CSNPs in *T. castaneum*
- To assess the *in vitro* and *in situ* antifungal and anti-aflatoxigenic activities of GEO, GEO-CSNPs, TEO and TEO-CSNPs against *A. flavus*

Materials and Methods

Insect rearing

The adult *C. serratus* and *T. castaneum* was collected from infested grains purchased from a local market and was maintained at 28±1°C temperature and 70-75 % relative humidity (RH); under a 16: 8h light: dark cycle for rearing the pests.

Molecular Identification of Stored Groundnut Pests

Genomic DNA was isolated from the abdominal tissue by using Qiagen DNeasy Blood and Tissue Kit (Germany). 1% Agarose Gel Electrophoresis was performed and the genomic DNA was detected in a Gel documentation system. DNA amplification of mt-COI gene was carried out with primers of forward and reverse in natures, COI gene: LCO1490 5'GGTCAACAAATCATAAAGATATTGG3' and HCO2198 5'TAAACTTCAGGGTG ACCAAAAAATCA3' respectively (Hebert *et al*., 2004). The PCR was carried out total volume of 50-µl reaction mixture consisted of 5 µl of extracted DNA, 1.5 mM MgCl 2 (Sigma, Bangalore), 0.2 mM dNTPs (Sigma, Bangalore), 1× reaction buffer (Sigma, Bangalore), 1.5 U Taq DNA polymerase (Sigma, Bangalore) and 0.3 µM of each primer. The amplified PCR product was visualized in 2% agarose gel electrophoresis (AGE; GENEI, Bangalore), and the image was documented with the gel documentation (Medic Care, India). The final product was stored at - 20 °C for further use. Sequencing was performed by using ABI 3500 XL Genetic Analyser with manufacturer's protocol of Chromos Biotech, Pvt. Ltd., Bangalore, India. The forward and reverse sequences were aligned pairwise by using CAP3. The sequence similarity available with NCBI database was identified by BLAST. The trimmed sequences were trimmed and edited using ClustalW and Bio Edit v.7.2.5 and submitted to National Center for Biotechnology Information (NCBI) GenBank.

Phylogenetic analysis

Phylogenetic analyses were constructed using MEGA v6 with neighbor joining (NJ) (Saitou and Nei, 1987) and maximum likehood (ML) methods (Pond *et al*., 2008).

Plant Material

Fresh garlic and turmeric rhizomes were obtained from the agricultural field of Sathyamangalam, Erode, India, located at 11.50'34" latitude, 77.24'44" longitude.

Essential oil extractions

The hydrodistillation method was used to obtain GEO and TEO in the Clevenger apparatus (Prakash *et al*., 2013).

Gas chromatography-Mass-spectrometry (GC-MS) analysis

The chemical profile of GEO and TEO essential oil was analyzed by GC-MS analysis. The GC apparatus used was a PerkinElmer equipped with a Turbo mass Gold mass spectrometer. Software was Turbo mass version 5.4.2.

Preparation of essential oil loaded Chitosan Nanoparticles

GEO-CSNPs and TEO-CSNPs were prepared through ionic-gelation technique following the protocol of Yoksan *et al*. (2010) with slight modification.

Characterization of essential oil loaded Chitosan Nanoparticles

Malvern Zeta sizer 3000HS (Malvern Instruments Ltd., UK) equipped with a He–Ne laser operating at 633 nm and 4.0 mW was used to perform dynamic light scattering (DLS) in order to measure the particle size, zeta potential, and polydispersity index (PDI). At room temperature, samples were sprayed with high purity water (1:100 v/v). The chemical structure of chitosan nanoparticles was analyzed using Fourier-transform infrared (FTIR) spectroscopy using a Shimadzu (IRAffinity) spectrometer with a wave number range of 400–4000 cm−1 and resolution of 4 cm−1 and 64 scans. Thermogravimetric analyser (TGA Instruments TGA Q20) and a standard platinum pan, thermal decomposition mechanisms were determined the thermal stability of chitosan nanoparticles. The X-ray diffraction (XRD) patterns of the samples were scanned over a 2θ range of 5–80◦ using an X-R diffractometer (Model-XMax, Oxford Instruments, Wiesbaden, Germany) with a step angle of 0.02◦ min− 1 and scan speed of 5◦ min− 1. Using TEM, the surface morphology and shape of freeze-dried chitosan nanoparticles were examined (ICON ANALYTICAL, FEI, QUANTA 200). Samples were glued onto an adhesive tape mounted on the specimen stub and particles were covered with a layer of platinum by Emitech sputter coater at 15 kV.

Insecticidal fumigant toxicity assay

The fumigant toxicity of GEO, TEO**,** GEO-CSNPs and TEO-CSNPs was tested against adults of *C. serratus* and *T. castaneum* by impregnated paper assay (Rajkumar *et al*., 2020) with slight modification.

Antifeedant activity

Antifeedant activity and alteration in nutritional physiology were estimated in accordance to (Shukla *et al*., 2011) with slight modifications.

Repellency test

The repellent assay of GEO, TEO**,** GEO-CSNPs and TEO-CSNPs against adults of *C. serratus* and *T. castaneum* was measured according to Zhang *et al*. (2011).

Acetylcholinesterase (AChE) Activity

AChE activity was estimated by colorimetric method as described by Lopez & Pascual-Villalobos, (2010).

Superoxide Dismutase Activity

SOD activity was observed by method described (Kiran *et al*., 2017).

Catalase activity

CAT activity was observed by method described (Ranjith *et al*., 2023).

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from adult *T. castaneum* using TRIZOL reagent following the manufacturer's protocol (Life Technologies, USA). The extracted RNA was treated with RNase-free DNA set (5 Prime GmbH, Germany) to remove the DN A contamination. The total RNA was quantified (Nanodrop 2000, Thermo Fisher Scientific, USA). First-strand cDNA was synthesized from total RNA using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio, USA) followed by Oligo (dt) (20) primer in accordance with the manufacturer's protocol.

Quantitative Real-time PCR was performed (QuantStudio 5 Real-Time PCR System, Applied Bio systems, Foster City, CA, USA). Real-time PCR reactions were carried out using 1X Maxima SYBR Green qPCR Master Mix, 300nM of forward and reverse primers (InR : Forward 5´- ACCCAAAGTGGTGAATAGCA-3' Reverse 5'-TCGTCCATCATAGAGCGTAA-3 Cyclin E: Forward 5´-GCGTACGGGTACGAAA ATGC-3' Reverse 5'- TTGGAATCGTCAGCGGACTC-3 4EBP: Forward 5´- GATGGC AAGACAAGTGAC-3' Reverse 5'- CGGAGTAGTGGAGTAGAG-3 FOXO : Forward 5´-TGCCCTGTTCGAGGTTTCCT-3' Reverse 5'-TGCCCTGTTCGAGGTTTCCT-3 Ribosomal proteinS3 : Forward 5´- CCGTCGTATTCGTGAATTGACTT-3' Reverse 5'-TCTAAGAGACTCTGCTTGTGCAATG-3), and 1.25ng of cDNA in a total volume of 18 µl. The following thermal profile was applied: 10 min at 950 C, and 40 cycles of 15s at 950 C and 60s at 600 C. The possible contaminations and primer dimers formation were discarded

by using no-template controls (NTCs). A standard curve with four points was obtained using the undiluted pool containing all cDNA samples and three 5-fold serial dilutions. All samples were duplicated. A melting curve analysis was performed to guarantee amplification of specific products. The values of quantification cycles (Cq) were obtained for each sample with the QuantStudio 5 software, Applied Biosystems, Foster City, CA, USA).

***In vitro* evaluation of antifungal and AFB_1 inhibitory potential**

In vitro antifungal and AFB_1 inhibitory efficacy of GEO, TEO, GEO-CSNPs and TEO-CSNPs was examined against the toxigenic strain of *A. flavus* [NFCCI (National Fungal Culture Collection of India) accession number: 4447] (Upadhyay *et al*., 2018). The AFB1 inhibitory efficacy of GEO, TEO, GEO-CSNPs and TEO-CSNPs was determined following the method modified of Chaudhari *et al*. (2020).

Mechanism of action: Biochemical

Effect of GEO-CSNPs and TEO- CSNPs on ergosterol content in the plasma membrane of *A. flavus*

The effects of different doses of GEO-CSNPs and TEO-CSNPs (0.25, 0.50, and 0.75 µL/ mL) on the ergosterol content in the plasma membrane of *A. flavus* were investigated following the method of Tian *et al*. (2012).

Effect of GEO-CSNPs and TEO-CSNPs on cellular ions

The estimation of cellular ions such as Ca2+, K+ and Mg2+ ions following the method of Helal *et al*. (2007) using atomic absorption spectrometry.

Effect of GEO-CSNPs and TEO- CSNPs on mitochondrial membrane potential (MMP)

MMP in *A. flavus* exposed to GEO-CSNPs and TEO-CSNPs was measured following the method of Tian *et al*. (2012).

Effect of GEO-CSNPs and TEO- CSNPs on the activity of the antioxidant enzyme

Enzymatic activity of Superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) enzyme activities were measured according to the methods described by Ranjith *et al*., (2023); Kumar *et al*., (2020). Estimation of protein was performed using Bradford's assay (Kumar *et al*., 2020).

***In situ* efficacy of GEO, TEO, GEO-CSNPs and TEO-CSNPs as antifungal and anti-aflatoxigenic in model food system (*Arachis hypogea L.*)**

In situ efficacy of antifungal and anti-aflatoxigenic analysis of groundnut seeds was performed by direct plating method following Kedia *et al*. (2014).

Efficacy of GEO, TEO, GEO-CSNPs and TEO-CSNPs against lipid peroxidation in groundnut seeds

Lipid peroxidation inhibition in GEO, TEO, GEO-CSNPs and TEO-CSNPs fumigated groundnut seeds was recorded in terms of malondialdehyde (MDA) content following protocol of Chaudhari *et al*, (2021) with slight modifications.

Statistical analysis

All the experiments in the present investigation were performed in triplicate and the data shown are the mean ± SE. The results were subjected to a one-way analysis of variance ($P \leq 0.05$) and Tukey's multiple-range tests to identify significant differences in the comparison of means. The data were analyzed using SPSS program version 16.0 for Windows (SPSS Inc., IBM Corp).

Results and Discussion

Molecular identification based on COI gene

The morphological identification indicated that the insect pests belong to Coleoptera order. The COI gene of *C. serratus* and *T. castaneum* species was sequenced species were submitted to the BLAST for performing similarity search for matching the sequence to the already existing data in the NCBI database. The sequenced showed 99.66%- 100% identity with *C. serratus* and *T. castaneum*. The sequence of COI gene of *C. serratus* was submitted to GenBank.

Chemical Composition of essential oil GEO and TEO

The GEO essential oil GC–MS analysis identified 19 volatile compounds and major components belongs to the terpens and monoterpenes classes, such as allyl methyl tri-sulfide (23.10 %), diallyl sulfide (19.47 %), c-sitosterol (11.15 %), benzyl alcohol (9.79 %) and diallyl tetrasulfide (8.38 %) which represented 71.89 % of the GEO components. The TEO essential oil GC–MS analysis 28 volatile compounds and major components belong to the terpenes and monoterpenes classes, such as α-turmerone (42.6 %), β-turmerone (14.0 %), and ar-turmerone 12.9 %), α -phellandrene (5.7 %) and 1,8-cineole (2.4 %) which represented 92.6 % of the TEO components.

Characterization of essential oil loaded Chitosan Nanoparticles

The GEO-CSNPs and TEO-CSNPs were prepared in two steps method. GEO and TEO droplet formation in chitosan solution used the oil-in-water emulsion approach. TEM image of GEO-CSNPs and TEO-CSNPs observed particles are spherical, with an 64-70 nm and 88–120 nm size range. A mean diameter of chitosan NPs without GEO and TEO showed

46.7 nm and GEO-CSNPs and TEO-CSNPs shows 88.7nm 132.4 nm. From the observation, TEO-CSNPs had negative surface charges ranging from -35.70 to -21.14 mV ZP value besides GEO-CSNPs had positive surface charges ranging from +20.4 to 35.7 mV. Crystallinity structure of GEO-CSNPs showed broad peaks with reduced intensity in the 2θ = 20º and TEO-CSNPs showed 2θ = 22º. FTIR spectra of chitosan and GEO peaks have seemed in the spectrum of GEO-CSNPs at the same wave number. Similarly, chitosan and TEO peaks have seemed in the spectrum of TEO-CSNPs at the same wave number.

Antioxidant activity of GEO-CSNPs and TEO-CSNPs

The antioxidant potential of GEO-CSNPs and TEO-CSNPs as determined by DPPH• and ABTS•+ assays. TEO-CSNPs exhibited intense free radical scavenging activity (IC_{50} value 0.97 and 0.66 µL/mL) in comparison with TEO (IC_{50} value 1.12 and 0.94 µL/mL), respectively and GEO and GEO-CSNPs were 13.5 – 42.17 % and 13.7 – 67.58 %, respectively.

Fumigant toxicity of essential oil and oil loaded chitosan nanoparticle

The mortality percentage of *C. serratus* and *T. castaneum* was higher when exposed to GEO-CSNPs than GEO. Similarly, TEO-CSNPs exposed concentration significant differences were noted than TEO. The LC_{50} and LC_{90} value obtained was found to be significantly effective in GEO-CSNPs *C. serratus* (24.32; 58.16µL/L air); *T. castaneum* (27.12; 59.34 µL/L air) when compared to pure GEO *C. serratus*, (44.35; 72.35 µL/L air), and *T. castaneum* (49.68; 78.65 µL/L air). In addition, for TEO-CSNPs were noted significant effective fumigant toxicity (28.61; 57.47µL/L air), and *T. castaneum* (34.79; 66.45µL/L air) when compared to pure TEO *C. serratus*, (56.48; 98.35µL/L air), and *T. castaneum* (62.94; 101.85µL/L air).

Repellent activity

GEO-CSNPs significantly possessed repellent activity against *C. serratus* and *T. castaneum* then GEO, 100 nL/cm2, showed a high repellency percentage activity of 92.6%, and 89.6% against adults of *C. serratus* and *T. castaneum.* TEO-loaded chitosan NPs, 100 nL/cm2, showed a high repellency percentage activity of 96.0%, and 92.0% against adults of *C. serratus* and *T. castaneum.*

Antifeedant activity

At LC_{50} and absolute toxicity concentration of GEO and GEO-CSNPs against adults of *C. serratus* and *T. castaneum* caused 100% feeding deterrent index (FDI). Weight loss of 6.57% and 8.79% were recorded in control groundnut infested with *C. serratus* and *T.*

castaneum. Sub lethal LC_{50} and absolute toxicity concentration of GEO and GEO-CSNPs significantly protected groundnut from insect pest infestation until the storage period. At LC50 and absolute toxicity concentration of TEO and TEO-CSNPs caused 100% feeding deterrent index (FDI) against both adults of *C. serratus* and *T. castaneum* and significantly protected groundnut grains from insect pest infestation until the storage period

Acetylcholinesterase activity

A dose-dependent inhibition of AChE activity was observed in *C. serratus* and *T. castaneum* exposed to GEO and GEO-CSNPs. At LC_{50} and sub lethal of LC_{50} dose, maximum inhibitions of AChE activity (52.43% and 37.71% respectively, for *C. serratus* and 37.80% and 31.29% respectively, for *T. castaneum*) were observed in both insect pest exposed to GEO-CSNPs for 24 h. Besides, Maximum inhibitions of AChE activity (75.67% and 35.74% respectively, for *C. serratus* and 69.87% and 28.74% respectively, for *T. castaneum*) were observed in LC_{50} and sub lethal of LC_{50} dose of TEO-CSNPs.

Antioxidant enzyme activity

SOD activity showed significant elevation in *C. serratus*, and *T. castaneum*, treated with GEO and GEO-CSNPs (16.52 and 19.15) and (26.21 % and 27.21%) than control. Significant decrease in CAT activity (4.99 % and 4.03 %) and (4.34 % and 3.67 %) treated to GEO and GEO-CSNPs than control. Similarly, SOD activity showed significant elevation in *C. serratus*, and *T. castaneum*, treated with TEO and TEO- CSNPs (26.21 % and 32.21%) and (16.52 and 20.15) than control. Significant decrease in CAT activity (4.21 % and 3.95 %) and (4.99 % and 4.03 %) treated to TEO and TEO-CSNPs than control.

Expression levels of growth-related genes

The two positively regulated genes were InR, and CyclinE; the two negatively regulated genes were 4EBP and FOXO were selected to determine their expression levels and relation with the body size of *T. castaneum* expression levels of InR, and CyclinE were significantly decreased in the LC_{20} and LC_{50} treatment groups when compared to those in the control group. The LC_{20} and LC_{50} treatments showed significantly increased expression levels of 4EBP, and FOXO, which is yet another reason for the change in the body size of *T. castaneum*.

***In vitro* antifungal and aflatoxin B_1 activity**

According to these findings, GEO-CSNPs complete inhibition of fungal growth at 1 µL/mL compared to GEO 2.0 µL/mL. In contrast, the average dry weight significantly reduced after increasing the concentration. Additionally, at a concentration of 0.75 µL/mL

GEO-CSNPs entirely inhibited the AFB_1 production, then GEO at 1.50 µL/mL. Similarly, TEO-CSNPs complete inhibition of fungal growth at 0.75 µL/mL compared then TEO 1.50 µL/mL, moreover at a concentration of 0.50 µL/mL TEO-CSNPs entirely inhibited the AFB_1 production, then TEO 1.25 µL/mL.

Antifungal and anti-aflatoxigenic mechanism of GEO-CSNPs and TEO-CSNPs

The percentage reduction in ergosterol content was observed to be 28.45, 44.36, and 52.37 %, treated at 0.25, 0.50, and 0.75 µL/mL of GEO-CSNPs, respectively. Also, 31.25, 49.58 and 58.36% reduction in ergosterol content was observed at 0.20, 0.40, and 0.60 µL/mL of TEO-CSNPs, respectively. GEO-CSNPs observed ions leakage analysis result indicates a significant enhancement in the leakage of Ca^{2+}, K^{+}, and Mg^{2+}, which are vital cellular ions, compared to the control group. Similarly, TEO-CSNPs showed a significant enhancement in the leakage of C Ca^{2+}, K^{+}, and Mg^{2+}. Different concentrations *viz.*, MIC and 2 MIC of GEO-CSNPs and TEO-CSNPs were determined by Rhodamine 123 and illustrates the percentage inhibition of fluorescence intensity of Rhodamine 123 dye. GEO-CSNPs at a concentration of 0.75 µL/mL showed a significantly enhanced in anti-oxidative defense systems such as SOD activity (24.36 %), CAT activity (19.24 %), GR activity (37.25 %) in *A. flavus*, compared to control. In addition, TEO-CSNPs showed a SOD activity (24.36 %), CAT activity (19.24 %), GR activity (37.25 %) in *A. flavus* at a concentration of 0.75 µL/mL.

***In situ* antifungal and AFB_1 inhibitory potential of GEO-CSNPs and TEO-CSNPs**

At the MIC and 2MIC doses, GEO-CSNPs exhibited better protection (85.23 % and 93.02 %) against fungal contamination, then GEO (41.75 % and 67.03 %), respectively. Also, 96.18% and 87.12% level protection were observed in TEO-CSNPs at the MIC and 2MIC doses, then TEO (53.14% and 72.43 %), respectively.

Lipid peroxidation inhibition of GEO-CSNPs and TEO-CSNPs

GEO-CSNPs exhibited maximum inhibition of lipid peroxidation, as MDA level was below 0.086 and 0.04 µM kg^{-1} at MIC and 2MIC doses, respectively than GEO. TEO-CSNPs exhibited maximum inhibition of lipid peroxidation, as MDA level was below 0.066 and 0.021 µM kg^{-1} at MIC and 2MIC doses, respectively.

Conclusion

As the application of EOs in food safety is problematic due to their low solubility in water, encapsulation techniques have been introduced in recent years as efficient means to increase their stability in aqueous media. In our present study, garlic and turmeric-loaded chitosan nanoparticles were successfully prepared by ionic gelation of emulsion technique

using STPP with considerably high encapsulation efficiency. The particles were spherical with different size range ratios of chitosan/GEO/TEO. Furthermore, in-vitro release studies indicated that the content of garlic and turmeric-loaded chitosan NPs influenced its release rate from the chitosan nanoparticles at different pH buffer media. Garlic and turmeric essential oil loaded chitosan nanoparticle enhanced DPPH radical scavenging activities. In fumigant toxicity, garlic and turmeric-loaded chitosan nanoparticles have a stronger insecticidal activity against the stored grain insect pest *C. serratus*, and *T. castaneum* compared to pure garlic and turmeric essential oil. Garlic and turmeric-loaded chitosan nanoparticles showed a significant level of *AChE* inhibition and antioxidant enzyme system activity against *C. serratus*, and *T. castaneum*. Garlic essential loaded chitosan nanoparticles showed a significantly regulated growth genes, which would negatively affect the population dynamics and stability of *T. castaneum*. Nanoencapsulated garlic and turmeric-loaded chitosan nanoparticles showed enhanced efficacy of antifungal and aflatoxin B_1 inhibitory activity compared to free garlic and turmeric essential oil. The antifungal and anti-AFB_1 mode of action of GEO-CSNPs was related to the impairment in cellular constituents of the plasma membrane and a reduction in mitochondrial membrane potential, and modifying the antioxidant defense mechanism, respectively. Overall findings concluded that garlic and turmeric-loaded chitosan nanoparticles developing an innovative design and safer EO based nano-insecticides that can be considered promising indications for the management of stored product insect pest control and utilized as a novel food preservative of stored grains and other food products to extend their shelf-life from *A. flavus* infestation and AFB_1 contamination.

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